Peer Review
Experimental Toxicology Division
NHEERL

Environmental and Genetic Interactions in Hypertensive Rats: Oxidative Stress as a Common Susceptibility Attribute for Non-cancer Risks

SUSCEPTIBILITY, ALLERGY & ASTHMA

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INTRODUCTION

Identifying genetic and environmental risk factors governing variations in susceptibility to a disease or its exacerbation, have been at the forefront of biomedical research field in the post-genomic era because of the available technologies. A sizable research effort is being made by private as well as governmental sectors with respect to determining genetic variations in humans genes and their relationships to disease susceptibility. This provides a great opportunity for us to develop parallel toxicological approaches that allow identification of risk factors of environmentally-induced diseases in animals.

Reducing uncertainty in risk assessment by identifying mechanisms of susceptibility is one of the primary research goals of the Human Health Research program of ORD.

Oxidative stress has been identified as a major contributor to susceptibility to a variety of diseases of many organ systems, such as cardiovascular disease (CVD), cigarette smoke-induced chronic obstructive pulmonary disease (COPD), asthma, Parkinson's disease, and Alzheimer's disease, as well as diseases of the reproductive and ocular systems. Oxidative stress has also been implicated in aging.

The proposed program project is designed to address the role of oxidative stress in susceptibility. We posed these primary questions: 1) Is oxidative stress a common susceptibility attribute or a risk factor for a variety of toxic insults affecting diverse organ systems? 2) And if so, what toxicological approaches will allow us to investigate its role and contribution in the exacerbation of environmental effects?

Our research program takes a comprehensive collaborative research approach using a rat model that demonstrates phenotypic susceptibility to oxidative stress and cardiovascular disease.

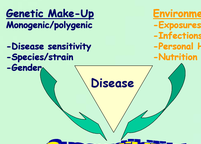
PROGRAM PROJECT GOALS

The Spontaneously Hypertensive (SH) rat can be validated as a relevant model of oxidative stress Phenotype in human. The SH rat has been shown to exhibit systemic oxidative stress and cardiovascular disease with an etiology remarkably similar to that of the human disease and disease-associated oxidative stress. Tissue levels of antioxidants have been shown to be lower with increased oxidized materials. The genetically similar, parental Wistar-Kyoto (WKY) rat is used as a control because it is known not to express the oxidative phenotype, or a cardiac disease. The genetic basis for susceptibility is unknown.

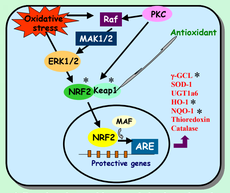
Using the SH rat as a relevant animal model of oxidative stress/disease, this program project hopes to provide a semiquantitative estimate of oxidative stress/disease-related susceptibility, determine the role of oxidative stress, and elucidate the environmental and genetic mechanisms, such that strategies can be developed for reducing uncertainty in estimating human risk from non-carcinogenic toxicants.

OVERARCHING HYPOTHESIS

Systemic oxidative stress is a common susceptibility attribute for effects of toxicants. Genetic predisposition to oxidative stress is a risk factor for increased susceptibility to environmental exposures

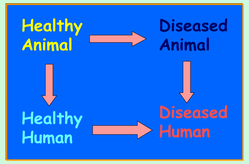


ROLE OF NRF2 IN OXIDATIVE STRESSS-MEDIATED SIGNALING

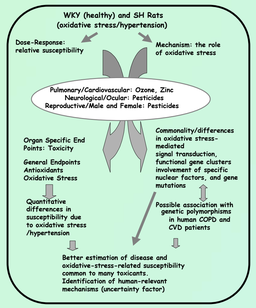


* Genetic polymorphisms are being investigated

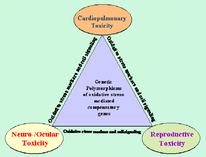
TOXICOLOGICAL PARADIGM FOR USE OF SUSCEPTIBLE DISEASE MODELS



FLOW CHART
HOW STUDIES WILL LEAD TO BETTER RISK ASSESSMENT



Schematic of Research Approach



SPECIFIC AIMS

1. Validate SH rat as a model of increased susceptibility due to oxidative stress/disease and establish oxidative stress biomarkers
2. Investigate comparative toxicities in SH and WKY rats of a variety of toxicants affecting different organ systems
3. Develop effective antioxidant treatment regimens that alleviate oxidative stress in SH rats
4. Determine the effectiveness of reduced oxidative stress in protecting SH rats from toxic effects
5. Evaluate signaling pathways, transcriptional regulation, and expression profiling to better understand biological mechanisms
6. Identify presence/absence of known human single nucleotide polymorphisms (SNPs) in SH rats that confer susceptibility and model validity

EXPERIMENTAL APPROACH AND METHODS

Cardiopulmonary toxicity	Neurotoxicity	Ocular toxicity	Reproductive toxicity
Lead EPA PIs: Urmila Kodavanti, Peter Gilmour, Penn Watkinson, Gary Hatch	Robert MacPhail, Prasada Kodavanti, Bellina Veronesi	Andrew Geller, William Boyes	Jeff Welch, Sally Darney, Robert Zucker
Major collaborators: Jane Gallagher (HSD), Reeder Sams (HSD), Andy Ghio, (HSD), Thomas Illig (GSF), Steven Kleeberger (NIEHS)			
Toxicants: zinc, ozone	carbamates, organophosphates (fenthion, chlorpyrifos, diazinon, carbaryl, paraquat)	carbamates, organophosphates (fenthion, chlorpyrifos, diazinon, carbaryl, paraquat)	acrylamide, thiram molinate
Target organs: lung and heart	brain regions	eye	testes and ovary
Specific endpoints: lung injury markers, lung and cardiac pathology	behavioral, neurochemical neuropathological glial cell cultures	high illumination-mediated exacerbation of physiological and morphological effects	sperm motility zygote formation

Antioxidant intervention:
1-3 weeks treatment protocol for nacysteline, trolox, tempol, and ascorbate followed by toxicant exposure

Molecular endpoints common to all projects:
Affymetrix gene arrays, transcription factor, and antibody arrays for tissue expression profiles

cell signaling: MAP kinases, PKC-mediated signaling
nuclear factors: NFκB, NRF2/AP1
gene expressions: Phase II metabolism genes, oxidative stress response genes

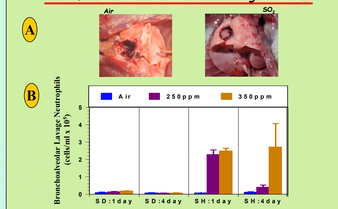
Protein/antioxidant/oxidant markers common to all projects:
plasma: α-1 Antiprotease, lipid peroxidation, total antioxidant capacity, aconitase
tissues: γ-GCL, NQO1, HO-1, GSH-S transferase, GSH peroxidase, α-1 antiprotease, lipid peroxidation

Genetic polymorphisms:
NRF2 gene and promoter, γ-GCL light and heavy subunits, HO-1, Apoprotein E, NQO1, GSTs, TNF, and markers of cardiovascular disease

Human Polymorphisms and cardiopulmonary disease association:
NRF2 and γ-GCL subunits

VALIDATION OF THE SH RAT AS A MODEL OF INCREASED SUSCEPTIBILITY AND OXIDATIVE STRESS

SH rats are more susceptible than SD rats to sulfur dioxide-induced chronic lung disease



Comparative pathological effects of SO_2 in SH and SD rats

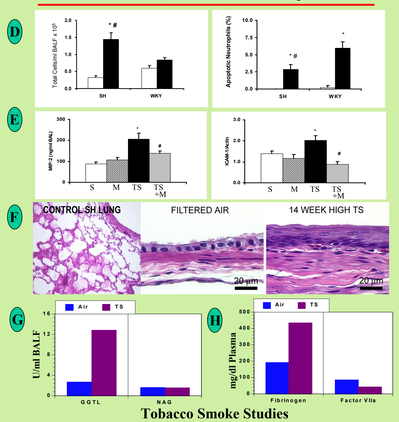
Histopathology Scores Indicating Strain Differences in SO_2-Induced Inflammation, Bronchiolar Hyperplasia, and Goblet Cells

SHR	Air (13)	SO_2-250ppm (13)	SO_2-350ppm (13)
Edema	2.2	1.9	2.0
Macrophages	1.2	1.7	1.6
Active inflammation	0.0	1.2	0.9
Bronchiolar hyperplasia	0.5	1.4	1.7
Goblet cells	1.1	2.3	2.3

SD	Air (13)	SO_2-250ppm (13)	SO_2-350ppm (13)
Edema	1.9	1.9	1.9
Macrophages	1.7	2.0	2.0
Active inflammation	0.5	1.0	0.9
Bronchiolar hyperplasia	1.1	1.1	1.2
Goblet cells	1.3	1.3	1.5

Sulfur Dioxide Studies

SH rats are more susceptible than SD and WKY rats to tobacco smoke-induced chronic lung disease



Tobacco Smoke Studies

Sulfur dioxide-induced* airways disease:

A) Significant air trapping in the lungs of SH rats
B) Twenty-seven times greater neutrophilic inflammation in SH rats when compared to SD rats
C) Greater goblet cell hyperplasia and mucus hypersecretion in SH than in SD rats

* 350 ppm SO_2x5h/dx4d

Tobacco smoke-induced chronic lung disease/Tobacco smoke as a model oxidant:

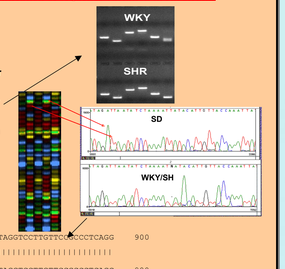
D) Significant inflammatory response in SH when compared to WKY rats but fewer numbers of apoptotic cells suggesting compromised ability to clear inflammatory cells recruited by tobacco smoke*
E) Pretreatment with SOD mimetic reduced tobacco smoke-induced inflammatory cytokine expression suggesting involvement of oxidative stress**
F) Parenchyma effluents are often present in control SH lungs indicating border-line pulmonary hypertension. Tobacco smoke induces dramatic mucus cell metaplasia following long-term exposure*
Γ) γ-Glutamyl transferase (GGTL), a marker for pre-cancerous changes, was significantly increased in BALF of SH rats where as n-acetyl glucosaminidase (NAG), a marker for macrophage activation was not changed***
H) Significant systemic changes occurred following acute exposure to tobacco smoke in SH rats, such as doubling of plasma fibrinogen and ~50% reduction in factor VII activity***

*: 60-70 mg/m³x6h/dx3d/wkx14 wks
** SOD mimetic, AEOL-10150, 5mg/kg, IT prior to ETS exposure.
TS, 78 mg/m³, 6h/dx2d. S=Saline, M=mimetic, TS=tobacco smoke
*** 60-70 mg/m³, 6h/dx2d

AN APPROACH TO IDENTIFYING OXIDATIVE STRESS AND DISEASE-ASSOCIATED HUMAN GENETIC POLYMORPHISM IN RAT MODELS

Sequencing rat NRF2 promoter, and identification of sequence variation in rat strains

1. Mouse NRF-2 promoter sequence known
2. Primers designed from mouse sequence for rat DNA amplification
3. PCR amplification of entire NRF-2 promoter region in overlapping fragments
4. Sequenced all promoter fragments for each rat strain
5. Align and compare strain sequences



SD 851 TCCGCCTTTAAGTTCTTGTCCCATCCCTAGGTCCTTGTTCCGCCCTCAGG 900
WKY/SH 851 TCCGCCTTTAAGTTCTTGTCCCGTCCCTAGGTCCTTGTTCCGCCCTCAGG 900

CONCLUSIONS AND IMPACT

Variations in human susceptibility to environmental insults necessitate consideration of most susceptible subgroups in risk analysis. The issue is which groups are to be considered susceptible, given the fact that there are a variety of diseases, age groups, and predispositions which may render humans susceptible to more than one insult.

This program proposal emphasizes consideration of oxidative stress as a common biological susceptibility attribute within human populations and animals that make them more susceptible to many types of environmental factors. We have validated a human-relevant rat model as a model of disease demonstrating oxidative stress. While genetics of susceptibility in this model may involve multiple markers which are yet to be identified, the disease progression and presence of systemic oxidative stress are highly similar to that of susceptible humans.

We have developed approaches to identify genetic polymorphisms that may be linked to oxidative stress and susceptibility to many types of environmental toxicants that affect diverse organ systems in a toxicant-specific manner, and subsequently cause a variety of diseases.

This program project will be crucial to developing approaches to incorporate oxidative stress as one common biological factor for enhancing susceptibility to a variety of environmental toxicants. This will also involve consideration of genetic polymorphisms as a prescreening tool for susceptibility in humans.

FUTURE DIRECTIONS

Since advanced molecular technology will allow us to simultaneously identify complex biological pathways, we propose to use this model to elucidate the role of oxidative stress in the predisposition and increased susceptibility to a disease.

Multiple target organ systems and toxicants will be evaluated with primary focus on what happens to oxidative stress responsive signaling, activation of transcription factors and gene expression, and how SH rats differ in terms of toxicity and involvement of oxidative stress.

We also propose to identify genetic polymorphisms that govern human susceptibility to oxidative stress in this rat model, with the rationale being validation of the model from a genetics perspective. We hope that availability of a biologically and genetically well-characterized model will facilitate understanding of the genetics in oxidative stress susceptibility and create more informative therapeutic approaches.

SOLVING AGENCY PROBLEMS